Pharmacognostic, Phytochemical and Pharmacological Investigation Of *Hypericum perforatum* and *Alternanthera sessilis* For Anticonvulsant Activity

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By

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1. INTRODUCTION

1.1 DEVELOPMENT OF HERBAL MEDICINE

Herbal medicine is a great and safe way to treat a variety of diseases. Modern methods of delivering herbal medicines, such as a novel drug discovery system with multi-component pharmacological effects, are being developed (Jun-ling Ren et.al., 2023). Because of advances in science and scientific equipment, we can now investigate many phytochemicals derived from herbal plants for the effective and successful treatment of a wide range of ailments. The majority of herbal plants contain pharmacological active phyto-constituents such as terpenoids, tannins, and flavonoids which have anti-oxidant and anti-inflammatory properties. (**Srivastava P., et.al., 2022).**

China, India, South America, Egypt, and other ancient civilizations continue to use herbal medicines to treat a variety of diseases. 7 **(Sharma A., et.al, 2008).**

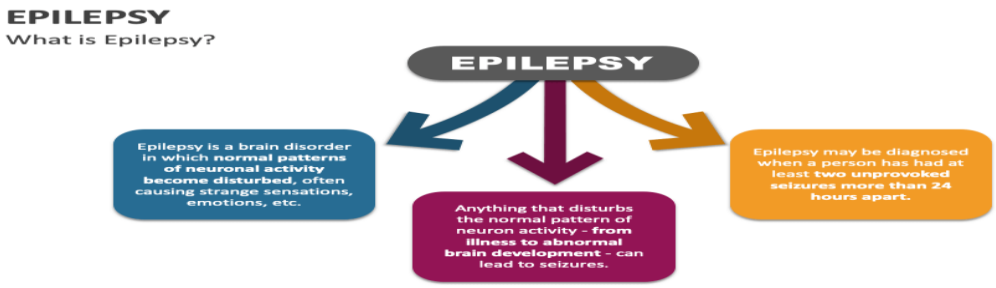
In India, approximately 25,000 licenced pharmacies of Indian medicines have registered approximately 1000 single drugs and 3000 compound formulations. Approximately 8000 medicinal plants are used in India's herbal industries. **(Kamboj, V.P., 2000).**

WHO guidelines must be followed by all herbal formulations. The primary goal of WHO guidelines is to define basic criteria for evaluating drug and herbal medicine quality control and safety efficacy. India is one of the top 12 biodiversity hotspots in the world., with approximately 15,000-20,000 having medicinal or pharmacological properties. Many Ayurvedic professionals use only 7,000-7,500 of these plants. About 600 plants used in the Siddha system of medicine, 700 in Ayurveda, and 700 in Unani. Herbal technology is one of the most profitable in India. India is an important supplier of herbal products, not only to meet domestic demand, but also to capitalize on the enormous market. The international specification for global suppliers of herbal medicines still requires the following aspects to be addressed: All medicinal plants in the Indian System of Medicine must be properly identified by a registered authority. Processing medicinal plants in a scientific, economical, and safe manner using methods similar to those used in modern drugs. Biological and clinical testing

to determine efficacy and safety; standardization to ensure uniformity.Combining medicinal plants should be used selectively in to facilitate analysis and apply quality control and standardization guidelines to herbal drug preparations. Documentation data for the research. These scientific elements of herbal medicine should be taken into account as they benefit to the long-term viability of the worldwide market. Approximately 80% of the world's population, according to WHO, utilizes herbal products for healthcare because modern medicine has few side effects and is economical.**(Sharma, A., 2008).**

1.2 DISEASE PROFILE

Epilepsy is a type of nervous disorder in which clusters of nerve cells or neurons in the brain suddenly function abnormally, resulting in unusual behaviour or emotions, sensations, or muscle spasms. A person can be either unconscious or conscious (Zahra Rabiei.,2017). Epileptic seizures can occur for a variety of reasons, including genetic, developmental, or acquired factors. Epilepsy has been diagnosed in approximately 50 million people worldwide. **(Fisher R.S., et.al., 2005).**


EPILEPSY
What is Epilepsy?
EPILEPSY
Epilepsy is a brain disorder in which normal patterns of neuronal activity become disturbed, often causing strange sensations, emotions, etc.
Anything that disturbs the normal pattern of neuron activity - from illness to abnormal brain development - can lead to seizures.
Epilepsy may be diagnosed when a person has had at least two unprovoked seizures more than 24 hours apart.

1.2.1 EPILEPTIC SEIZURE TYPES

Seizures are classified into two types:

A. Focal Seizures or Partial Seizures - A large portion of one hemisphere of the brain or only a small portion of a lobe is affected by focal seizures. The focus of the seizures is another term for it.

B. Generalized seizures - Generalized seizures primarily affect both hemispheres of the brain, causing affected individuals to become unconscious for a few seconds**(Zahra Rabiei., 2017).**

1.2.2 ANTIEPILEPTIC DRUGS ACTION MECHANISM

The mechanism of action of anti-epileptic drugs consists primarily of three steps:

• First, it reduces the electrical excitability of cell membranes, primarily by blocking sodium channels based on their use.

- Second, by increasing GABA's post-synaptic action by inhibit GABA transaminase enzyme or directly interact with GABA agonist.

• It prevents T-type calcium channels from opening (important in controlling absence seizures)**(Rang & Dale., 2003).**

1.3 PLANTS PROFILE

1.3.1 *Hypericum perforatum*

Merging medicinal plants should be used cautiously in to facilitate analysis and apply quality control and standardization criteria to herbal drug preparations. Documentation data for the research. These scientific facets of herbal medicine should be taken into account as they contribute to the long-term viability of the worldwide market. Approximately 80% of the world's population, according to WHO, depends on natural products for healthcare even though modern medicine has few side effects and is affordable.**(Joanne, B., et.al., 2001).**

*Hypericum perforatum*is primarily composed of several biologically active compounds that are responsible for treatment of various diseases. But due variation in genetic characters within species and/or adulteration, ecological factors, sample preparation and processing, and storage conditions such as light and harvesting time, the concentrations of these phyto-

chemicals frequently vary. Bioactive compounds are abundant in buds, blossoms, and twig tips. It has been investigated that about 20% of plant extract contains biologically active compounds..**(Ana., I.O., et.al., 2016).**

Scientific classification of *Hypericum perforatum*

Kingdom : Plantae
Subkingdom :Viridiplantae
Division :Tracheophyta
Sub-division :Spermatopytina
Class : Magnoliopsida
Order :Malpighiales
Family :Hypericaceae
Genus :*Hypericum*
Species : *Hypericum perforatum*

Figure 1. ***Hypericum perforatum***

1.3.2 *ALTERNANTHERA SESSILIS*

Multicellular joy plant, also known as Alternanthera sessilis. It's an established shrub whose young shoots and leaves are served as vegetables.In Sri Lanka, it is served as a soup. It has been used in China, Taiwan and India as a medicine for a long time. *Alternanthera sessilis* is a

member of the Amaranthaceae family and can be found throughout India's tropics and subtropics, reaching 1200m in the Himalayas. *A. sessilis*is an annual or perennial plant with several spreading branches and simple petiolate leaves. It's also embellished with small white colour flowers. It is also grown as a pot herb..**(The Wealth of India, 1948).**

Matyakshika is the Ayurvedic name for *A. Sessilis.* It has traditionally been used to cure ulcers, skin diseases, and problems with vitiated blood in Indian traditional medicine.**(Ayurvedic Pharmacopoeia of India, 1999).**

It's been used as a cholagogue (a substance that increases bile flow in the liver), an abortifacient (a substance that causes abortion), and a febrifuge for centuries (treats fever). Moreover, it is used to treat snakebites, boils, dysentery, diarrhoea, and wounds. It is used to treat acne and pimples on the skin **(A.K. Nadkarni, 2002)**. Plant stems that are herbaceous, weak, and cylindrical. Its branches radiate outward from the centre. The stem is yellowish-brown to light brown in colour. This plant has sessile leaves, a herbaceous, weak, cylindrical stem, and branches that spread from the base. The plant has no distinct odour or flavour. The fruits measure 1.5mm in length and have thick margins.. Flowers are white often tinged with pink in colour. As lateral rootlets, many roots emerge from the tap root. **(P. Pundareeksha Rao 2016).**

The plant contains polyphenols, terpenes, alkaloids, and carotenoids. It also contains the nutritional compounds vitamin C, E, and unsaturated fatty acids **(Chia Shing Hwong., 2022)** catechin, rutin, epicatechin, ellagic acid, quercetin, vanillic acid, ferulic acid,ethyl gallate, p-hydroxybenzoic acid and apigenin. were discovered in the plant through chromatographic analysis. **(Othman. A., et.al., 2016).**

Scientific Classification of *Alternanthera sessilis*

Kingdom : Plantae

Subkingdom :Tracheobionta

Division :Magnoliophyta

Sub division :Spermatopytina

Class : Magnoliopsida

Order : Caryophyllales

Family :Amarnanthera

Genus :*Alternanthera*

Species :*Alternanthera sessilis*

Figure 2.*Alternanthera sessilis*

2. LITERATURE REVIEW

Chinenye J., (2023) demonstrated anticonvulsant activity of aqueous extracts of aerial parts of Carallumadalzielii in chicks and rats models of seizures induced by pentylenetetrazole (PTZ) and strychnine (STN). According research on oral acute toxicity in mice, the LD50 was higher than 5000 mg/kg. Furthermore, the extract dose-dependently reduced the duration of tonic seizures and extended the latency/onset of myoclonic convulsions.

Rajinder Singh., et.al., (2023) studiedthe results demonstrated that the extract from Ficus benjamina figs prolonged seizure latency and attenuated the duration of tonic chronic convulsions. Additionally, the duration of the tonic extensor and flexor muscles to flexor ratio (E/F ratio) in an amount of the drug pattern was reduced significantly.

Anthony S., et.al., (2022) reported anticonvulsant activity of a hydro-ethanolic extract effects of *Ceiba pentandra*leaves extract (30-300 mg/kg, p.o.) on maximal electroshock (MES) and pentylenetetrazole models, 4-aminopyridine, strychnine, and picrotoxin induced seizures, and status epilepticus (lithium/pilocarpine) test. It reduces the frequency and duration of convulsions caused by pentylenetetrazole (PTZ) and picrotoxin.

Zahra Rabiei., (2017) presented information about epilepsy and its treatments, focusing on herbal remedies and introducing plants that are antiepileptic and outlining those plants' mechanisms of action.

Dinesh Y. Ganwande et al. (2017) When compared to saline, The extract of *Achyranthes aspera* Linn. reduced convulsions in mice induced by pentylenetetrazole (PTZ), picrotoxin, and bicuculline, but it did not protect against MES (maximal ectro shock) induced seizures.

Gaurav Gupta., et.al., (2014) reported the median fatal dose of morusin from M. alba plant extract was determined to be 20 mg/kg b.w. (LD50). Morusin delayed convulsion symptoms and tonic hind limb extension, shortened the duration of tonic-clonic convulsions, and decreased mortality in isoniazid and maximum electroshock convulsions.

Kar., DM., et.al., (2014)reported the onset of convulsions in maximal electroshock (MES), PTZ, strychnine, and picrotoxin-induced seizures was substantially (P 0.01) enhanced by Mussaendaphilippica leaves extract at 100 and 200 mg/kg in comparison with the control group.

Sadeghi. A., (2022) revealed Dried flowers like Echium amoenum and Hypericum perforatum have been utilized for centuries in traditional Iranian medicine to address mental illnesses. After

4 and 8 weeks respectively of therapy, there were no significant differences between the groups having fluoxetine extract and standard medication (p>0.05). Dry mouth was the only side effect noted, and it was attenuated in the preprocess group (p0.05) in both the second and eighth weeks.

Sengun. D.N., et.al., (2021) showed decreased activity of several antioxidant enzymes, including peroxidase, catalase, and glutathione). The connective tissue did not exhibit any significant alterations upon immunohistochemical analysis. Moreover, *H. perforatum* L. reported important chemopreventive properties.

Sofi S.H., et.al. (2020) revealed that an ethanol-induced gastric ulcer model, *Hypericum perforatum*was found to have hepatoprotective effects in rats. The beneficial effects of gastric inflammation were strikingly similar to those of esomeprazole, a commonly used antiulcer medication.

A.I. Oliveira., et.al., (2016) revealed that Traditional uses for *Hypericum perforatum* include t treatments of mild burns, anxiety, and mild to moderate depression. The chemistry, pharmacological activity, medication interactions, and side effects of the primary active phyto-constituents of H. perforatum are thoroughly discussed. Find out more about its neuroprotective abilities. H. perforatum extract and a number of its key chemical components have been shown in this regard to be capable of preventing the effects of toxic substances, either directly via neuroprotective processes or indirectly via anti-oxidant properties.

Arif-ullah Khan., et.al., (2011) revealed that in several isolated tissue preparations, the pharmacological effects of *H. perforatum* extract fractions was isolated in petroleum ether (HpPet), chloroform (HpCHCl3), ethylacetate (HpEtAc), and aqueous (HpAq), as well as its chemical constituents (hyperforin, hypericin, and hyperoside) were studied. Like papaverine, the HpCHCl3, HpEtAc, and Hp Aq fractions reduced both spontaneous and K+ (80mM)-induced contractions in the rabbit aorta. With the exception of HpEtAc, which did not produce a vasoconstrictor or vasodilator effect, all of the fractions had a vasoconstrictor and vasodilator effect HpCHCl3, HpEtAc, and HpAq stimulated the heart in guinea pig atria before inducing an inhibition similar to papaverine. HpPet, which acts similarly to verapamil, only suppresses the heart, whereas hyperforin, hypericin, and hyperoside have the same spasmolytic effect as verapamil.

Saddique.Z., et.al., (2010) revealed that the plant contains anthraquinone derivatives (naphthodianthrones), flavonoids, prenylated phloroglucinols, tannins, and volatile oil, according to the literature. Tetraketone and hyperforin are the primary antidepressant components of the plant. According to the literature, it has more antibacterial activity against gramme positive bacteria than gramme negative bacteria, and alcoholic extracts have more pronounced activity than aqueous extract.

Hosseinzadeh.H., et.al., (2005) Usingpentylenetetrazole (PTZ) and the maximal electroshock seizure, the anticonvulsant activity of *Hypericum perforatum* aerial parts (aqueous and ethanolic extracts) was investigated (MES and PTZ induced models). It delayed the symptoms of tonic convulsions in the PTZ model, but neither extract showed anticonvulsant activity in the MES model. Both extracts' anticonvulsant activity was inhibited by L-NAME and a nitric oxide (NO) synthase inhibitor.

Jiri Patocka., (2003) reported *Hypericum perforatum* extract have better safety and tolerability profiles than synthetic antidepressants . Six major natural compounds are isolated from a hydro-alcoholic extract of aeiral plant parts containing 60% ethanol or 80% v/v methanol: 1) naphthodianthrones; 2) naphthodianthrones; 3) naphthodianthrones; 4) naphthodianthrones phloroglucinols 2 Flavonoids (three) 4) Isoflavones 5) Phenylpropanes; and 6) Proanthocyanidins. The phyto-constituents primarily responsible for the plant's antidepressant activity are unknown, but the The non-nitrogen compound hyperforin is now the most important phyto-component for antidepressant activity.

Jain et.al., (2021) reported antioxidant and anti-gout properties of ethanolic extracts of *Alternanthera sessilis* and *Moringa oleifera* have. The ethanolic extract of *M. oleifera* is found to be significantly more potent than *A.sessilis*.

Gothai S., et.al., (2018) reported that Alternanthera sessilis leaf extract reduced considerably colon cancer cells in a time- and dose-dependent way when comparison to aerial and stem extracts. Analysis of leaf extract using GC-MS showed the presence of different antioxidant, anti-cancer, and anti-inflammatory components.

Othman. A, et.al., (2016) reportedethanolic and aqueous extracts of red *Alternanthera sessilis* (ASR) and green *Alternanthera sessilis* (ASG) were compared for nutritional compounds, anti-oxidant activity, and phenolic compounds. A colorimetric reduction using the Folin-

Ciocateumethod used to calculate the total phenolic content. To investigate phenolic compounds, High Performance Liquid Chromatography was used in conjunction with diode array and electrospray ionisation mass spectrometric detection. ASR was found to have significantly more protein, fat, carbohydrate, and minerals than ASG (p0.05). The anti-oxidant capacity of ASR and ASG ethanolic extracts is greater than that of water extracts (p0.05).

Myreddy J. et.al., (2014) reported Maximal electroshock (MES) induced convulsions in Groups I, II, III, IV, V, VI, VII, and VIII animals were used to investigate the anticonvulsant activity of an ethanolic extract of the total Alternanthera sessilis plant at 200 and 400 mg/kg b.w. Saline solution was provided to Group I, phenytoin (25 mg/kg body weight) was given to Group II, high and low doses of ethanolic extract (200 and 400 mg/kg) were offered to Groups III and IV, piracetam (200 mg/kg body weight) was provided to Group V, phenytoin and a combination of high and low doses of ethanolic extract have been given to Groups VI and VII, and both were given to Group VIII. Convulsions were significantly reduced by A.sessilis.

Thomas M. Walter., et.al., (2014) revealed the importance of *Alternanthera sessilis*Plant in traditional medicine of Siddha literatures. This plant is made of gold and belongs to the Kaya Kalap category (Panacea). It also contains anti-oxidants, sterol compounds, has a cooling effect on the eye and body, relieves neutritis, treats 96 types of eye diseases, and helps people live a disease-free, healthier life.

Niraimathi. K.L., et.al., (2013) reportedAlkaloids, tannins, ascorbic acid, carbohydrates, and proteins work as effective reducing and binding agents for the transfer of silver nitrate in to other nanoparticles in the Alternanthera sessilis leaf extract. Additionally, proteins and ascorbic acid were evaluated on the generated silver nanoparticles (AgNPs). In furthermore, they discovered that green synthesized AgNPs have anti-microbial and antioxidant properties.

Anitha R et.al., (2012) reported the pharmacological and clinical characteristics of the herb Alternanthera sessilis. It shows the existence of calcium oxalate crystals in the leaves and stalk. In the analysis of a material, long uniseriate trichomes measuring 1227.4–1431.9 m has been discovered. The stem maceration was characterised by the presence of macro-sclerids and scalariform thickness. The cambium layer in between fascicles is evident in the vascular bundle. Phenols, flavonoids, and saponins have been discovered in the phytochemical of the water preparation of the leaves of a A. sessilis plant.

Jalalpure S.S., et.al., (2008) showed Alternanthera sessilis Leaf extracts.'s to repair wounds. The leaves were extracted utilizing five separate solvents (petroleum ether, chloroform, acetone, methanol, and distilled water). Cup plate and turbidimetric approaches were used to evaluate antimicrobial activity. Findings from 200 g/ml chloroform extract of A. sessilis leaves have been substantial.

3. RESEARCH ENVISAGED

There is now a worldwide awareness of the importance of studying indigenous / traditional medicines using modern scientific methods. Existing anticonvulsant medications are in high demand, despite the fact that they do not treat all types of convulsive disorders. Long-term treatment causes severe side effects in many patients, including chronic toxicity, cognitive impairments, and sedation. I chose *Alternanthera sessilis* and *Hypericum perforatum* to improve the efficacy and side effect profile of anticonvulsant plants. According to a review of the literature, *Alternanthera sessilis* and *Hypericum perforatum* have a broad range of biological activity based on various chemical constituents such as wound healing, anti-microbial, antidiabetic, hepatoprotective, and so on.By the ayurvedic & chemical claim this plant having flavonoids, triterpenes & traditionally it is using for anticonvulsant activity so on the basis of above finding my aim to do this work is to extract and investigate its anticonvulsant effects of both plants.

4. PLAN OF WORK

- Literature survey of selected medicinal plant.
- Collection and Authentication of drug.
- Pharmacognostic (Morphological and Microscopical evaluation)
- Pharmacopoeial standards (Physio-chemical evaluation)
- Extraction of plant materials
- Phytochemical study

a) Preliminary phytochemical screening

b) Characterization of the active extracts of both plants by

- Chromatography or spectroscopy analysis for estimation of chemical constituents.
- Pharmacological evaluation of the extract
- In vivo anti-convulsant activity of active extracts of both plant by using suitable animal model.
- Biochemical estimation
  - Anti-oxidant activity
  - Lipid peroxidation
- Network pharmacology analysis to investigate bio-molecular approach of plant metabolites in brain disorder or epilepsy
  - Compound and gene ligation analysis
  - Gene ontology analysis

5. METHODOLOGY

Collection of plant material- will be collected from the neighbour area or market and will be authenticated.

Pharmacognostic evaluation-Pharmacognostic evaluation will be done for detailed study.

(A) Morphology- Color, odour, taste, shape, size, texture, fracture, and other characteristics of each plant would be determined and compared to standard text or museum samples.

(B) Microscopy- Detailed qualitative microscopical studies would be carried out on both mention part of each plants. Micrometric determination, i.e. both plants would be measured for length and width of vessel and fibres, diameter of starch grains, and calcium oxalate crystals.

(C) Pharmacopoeial standards-

Numerous physical and chemical parameters in compliance to W.H.O. guidelines would be determined for various extracts. methodologies for detecting the moisture content involve total ash, acid-insoluble ash, and water-soluble ash, as well as strategies for determining extractive values including alcohol and water-soluble extractive values.

D**) Phytochemical investigation-**

The qualitative phytochemical compounds would be detected by performing respective chemical tests for both plants while compounds characterization would be done by modern chromatography and spectroscopic methods.

E) Pharmacological Screening-

Anti-convulsant Model

Technique for the 4-aminopyridine-induced convulsion test

Seven groups were utilized to group the animals. Distilled water (10ml/kg p.o.) was administered to one group (n=8) as the transportation. Three groups (n=6) received oral sodium valporate dosages of 100, 300, and 600 mg/kg. The extract was administered orally to the following three groups (n=8) in varying amounts. A before the dosage of 4-aminopyridine (14 mg/kg) was reconstituted in the diluent and administered subcutaneous

injection into the rats to cause tonic convulsions 60 minutes just after treatments. To calculate each mouse's latency to extraction and time of death, mice were examined for 30 minutes. The ability of an extract to significantly prolong latency and/or survival is the anticonvulsant activity set of criteria.

F) Biochemical Estimation

- **Anti-oxidant evaluation of both plants**

Seven groups were utilized to group the animals. One group (n=8) administered distilled water (10ml/kg p.o.) as a solvent. Taking sodium directly Weighed samples and the reference pharmaceutical (L-ascorbic acid) are dissolved in ethanol to a concentration of 1 mg/ml. Using a serial dilution technique, the extracts were then divided into four concentrations (125, 250, 500, and 1000 mg/ml), as well as the L-ascorbic and DPPH have been weighed and dissolved in ethanol to generate a 0.1 mM solution.Finally, each test tube containing 2ml of the sample sol was replaced to 2ml of 0.1 mM DPPH solution using a micropipette. The precipitate volume was 4ml. Afterwards when, the test tube was left in a darkened area for 30 minutes to encourage the reaction to complete. Additionally, DPPH and an equivalent volume of ethanol have been added to a neutral test tube. Each test tube's absorbance was measured at 517 nm by only a UV spectrophotometer.

Percentage inhibition is calculated –

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}$$

We estimated the IC50 value from a plot of % suppression against the log concentration of sample extract, which reflects the concentration of a sample required to neutralize 50% of the DPPH free radicals.**(Khatun M. et.al., 2021).**

- **Lipid per-oxidation inhibitory activity**

60 minutes of aqueous extract at various concentrations, finally 60 minutes of 1mM H2O2. The cells are then homogenized in 1.15% KCl after already being washed in cool PBS. 100 l of cell lysates and 0.2 ml of 8.1% SDS are merged in the samples. 1.5 ml of 20% acetic acid and 0.8% TBA, will each calibrated to pH 3.5, have been used. After that, the mixture is heated for 120 minutes at 95 C after already being adjusted to an entire amount of 4.0 ml

with distilled water. Each sample was then given time to reach room temperature before even being addition to 5.0 ml of a 15:1 v/v n-butanol/pyridine mixture and vigorously shaken. The supernatant fraction was isolated and the absorbance at 532 nm was measured after 10 minutes of centrifugation at 1500 rpm. To evaluate the inhibitory activity of lipid peroxidation, the IC50 value was used.**(Lee S.E. et.al., 2003).**

G) Network pharmacology analysis to investigate bio-molecular approach of plant metabolites in brain disorder or epilepsy

- Compound and gene ligation analysis
- Gene ontology analysis

6. EXPECTED OUTCOMES

In an effort to improve the efficacy and quality of Indian medicinal plants, I chose *Hypericum perforatum* and *Alternanthera sessilis* for anticonvulsant activity based on ayurvedic and chemical claims. Hydro-alcoholic extraction is used for both plant materials. Phytochemical screening tests were performed on freshly prepared extracts of both plants to detect various constituents. The compounds are then subjected to various analytical techniques. The extract's anticonvulsant activity is then determined. Extensive biomolecular research is being conducted, however, to determine plant's precise mechanism, active principles, and safety profile as a convulsive disorder treatment.

7. TIME LINE

Objectives	1-6	6-12	12-18	18-24	24-30	30-36
Collection and authentication of medicinal plants						
Preparation of alcoholic extracts						
Qualitative and quantitative phytochemical analysis of prepared extracts using modern analytical techniques such as UV spectrophotometer, HPTLC, LC-MS/GC-MS						
In-vivo anti-convulsant activity of prepared extracts						
Exploration of the multi-targeted therapeutic role of medicinal plants using Network pharmacology, Writing and publications						

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